Maple Sirup

XXII. Controlled Fermentation of Buddy Maple Sirup

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SUMMARY

The unpleasant flavor in buddy maple sirup can be removed by fermentation with *Pseudomonas geniculata* strain 4. The sirup must be diluted to aproximately 20° Brix for most effective removal of the buddy flavor, which occurs on incubation of inoculated dilute sirup for 24 or 48 hr at 27°C. Pilot-plant studies with volumes of dilute sirup ranging from 40 to 700 gallons have shown the feasibility of this procedure. The amino acid contents of buddy sirup were reduced by fermentation with *Ps. geniculata.* Leucine, methionine, aspartic acid, and threonine are more completely utilized than the other amino acids. *Leuconostoc mesenteroides* has been tentatively identified in buddy sirup and sap as a contaminant responsible for the development of a ropy or slimy condition. *L. mesenteroides* grew better in buddy sap than in normal sap, and viscosity and ropiness developed only in the buddy sap. *Ps. geniculata* grew better in normal sap than in buddy sap.

INTRODUCTION AND LITERATURE REVIEW

Sirup prepared from the sap of the sugar maple tree normally has a characteristic maple flavor. However, when climatic conditions cause the tree to come out of dormancy, a circumstance usually accompanied by a noticeable development of the buds, the composition of the sap changes. Sirup prepared from this sap possesses an unpleasant flavor and aroma and is known as "buddy" sirup. It has no value for table use. Although buddy sirup is usually produced late in the season, it may be produced at other periods of sap flow and without noticeable physical changes in the tree. This sap is not easily distinguishable from normal sap by its flavor, odor, or chemical tests, since the buddy flavor is not present in the sap but results during the heat evaporation of sap to sirup. On rare occasions, such as occurred in 1963 in some areas, buddy sap has been produced at the very beginning of the season.

In the past, when the weather became warm enough to induce the production of buddy sap, many tapholes "dried out," or stopped leaking sap, because the excessive growth of bacteria plugged the tissue tubules (Naghski and Willits, 1955). The producer also stopped collecting sap after the first batch of buddy sirup came through the evaporator. With the recent advent of paraformaldehyde pellets, contamination of the taphole is reduced and the sap can run for a longer period. Furthermore, there is a tendency for evaporator plants to process greater volumes of sap from a variety of sugarbushes some of which may be producing buddy sap. It is possible, therefore, for an evaporator plant to process quantities of buddy sap into unacceptable sirup. A previous publication (Wasserman and Willits, 1961) described a method for the conversion of buddy maple sap into normal sirup utilizing the controlled growth of *Pseudomonas geniculata* in the buddy sap. No information was presented, however, on the recovery of buddy sirup.

This paper describes a method of treating buddy sirup to obtain a product having the characteristic maple flavor and free of the buddy odor and flavor.

MATERIALS AND METHODS

Materials. *Buddy maple sirup.* The preliminary laboratory experiments were carried out with samples of buddy maple sirup submitted for analysis by producers of Pennsylvania and New York. The large pilot-plant studies were made with commercial sirup prepared in the conventional manner.

Culture. The organism used was *Pseudomonas geniculata* strain 4, previously described as having strong maple-flavor-inducing properties when grown in maple sap (Willits *et al.,* 1961). This organism was also used in the controlled fermentation of buddy sap to obtain normal sirup (Wasserman and Willits, 1961). The cells were grown in a medium consisting of 0.5% Difco (no endorsement implied) yeast extract in normal maple sap. Two-hundred-ml quantities of the medium were sterilized in 1-L Roux bottles at 15 psig in the autoclave. The inoculum for sirup fermentation was prepared from 24-hr cultures of *Ps. geniculata* grown on slants of Difco tryptone-glucose-yeast extract (TGE) agar or Difco Pseudomonas agar F. The cells were aseptically scraped from the slants and suspended either in sterile distilled water or in the sap-yeast extract medium. A sufficient amount of the concentrated cell suspension was transferred to the medium in the Roux bottles to produce just visible turbidity, although the number of cells used for the inoculum was found not to be critical. The inoculated bottles were incubated at 28–30°C in a horizontal position, providing greater surface area and, hence, greater growth. The bottles were shaken vigorously occasionally, to resuspend sedimented cells and to bring fresh oxygen into solution. After 72 hr of growth the cells were separated by centrifugation and resuspended in a small volume of sterile distilled water. The number of viable organisms in the concentrated cell suspensions was determined by standard plate counting techniques. Counts were made on Difco TGE after incubation for 48 hr at 29°C.

Procedures. In the laboratory, experiments were carried out in sterile 5-gal. carboys. The sirup media, treated as described in the Results and Discussion, were inoculated with an amount of the concentrated suspension of cells to give a viable count of $3\text{–}5 \times 10^6$ bacteria per ml. The inoculated sirup was incubated at room temperature (about 27°C) for the appropriate periods, and the dilute sirups were then concentrated to standard sirup (65.5° Brix) in a steam kettle.

Pilot-plant experiments were conducted at a commercial sap evaporator plant. Depending on the quantity of buddy sirup to be processed, 55-gallon drums, 500-gallon stock watering tanks, or a 1000-gallon tank truck were used. The experiments were carried out under field conditions, and no attempt was made to follow sterile procedures, but the work was done under conditions as sanitary as possible. The clean 55-gallon drums and stock watering tanks were scalded with flowing steam at atmospheric pressure for approximately 10 min, then washed with a solution of household hypochlorite bleach diluted 1:1 with water. The hypochlorite solution was held in the drums and tanks for 2 hr with intermittent agitation, then the containers were rinsed with two separate portions of water. The open tanks were covered with sheets of plastic material.

Maple flavor and the "buddy" taste in the sirups were evaluated by taste panels composed of people working with maple sirup and familiar with its taste.

RESULTS AND DISCUSSION

Maple sirup contains 65.5–66.5° sugar as sucrose. In such a concentration, *Ps. geniculata* will not grow and the buddy flavor remains in sirup inoculated with the organism. Preliminary experiments were carried out in which the buddy sirup was diluted arbitrarily with three volumes of water prior to inoculation. After 48 hr of incubation at 27°C the dilute solutions were reconcentrated to sirup in which the undesirable buddy flavor was no longer present. However, in some trials the dilute sirups became viscous after 48 hr of incubation, and sirup made from these solutions was ropy. Microscopic examination of the dilute sirups showed the presence of organisms other than the added *Pseudomonas.* These adventitious organisms were also found in undiluted sirup. The contaminants are considered further in another section of this paper.

To eliminate the activity of the contaminating organisms the effect of heat treatment of the sirup was investigated, with the results shown in Table 1. The dilute sirup, brought to a boil and held there for 5 min, did not become viscous or ropy after 5 days of incubation at 27°C, whereas the unheated aliquot of the dilute sirup began to thicken after about 30 hr of incubation and was very viscous after 48 hr.

Table 1. Effect of heat treatment on the development of viscosity and ropiness in dilute buddy maple sirup.

Treatment	Time[a]	Observations
Sirup heated 5 min at boiling		
	30 hr	Normal-appearing
	48 hr	No change
	120 hr	No change
Sirup not heated		
	30 hr	Appearance of viscosity
	48 hr	Very viscous
	120 hr	Very viscous, but not ropy

[a] Time of incubation following inoculation with *Ps. geniculata.*

Boiling the dilute sirup assures treatment of the diluent water as well as the sirup, but the increased volume poses a problem. So that the volume of material to be heat-treated would be kept small, undiluted sirup was heated to the boiling point (3.9°C above the temperature of boiling water) and held for 5 min before being diluted with 3 volumes of water. Sirup treated in this manner behaved like the sirup heated after dilution. Furthermore, more rapid cooling was obtained by using cold dilution water. However, the water must be of low microbial count (potable) and handled and stored in sanitary containers.

The heat treatment of the whole or diluted sirup does not affect the activity of the *Pseudomonas* on the buddy flavor. Table 2 shows that growth of *Pseudomonas* for 48 hr at 27° in both heat-treated and untreated dilute sirups resulted in removal of the buddy flavor in the two sirups, while the flavor of

Table 2. Effect of heat treatment of dilute buddy sirup on *Ps. geniculata* activity in removing buddy flavor.

Treatment	Flavor[a]
Sirup heated 5 min at boiling	Not buddy
Sirup not heated	Not buddy
Sirup not heated and not inoculated	Very buddy, became ropy after 72 hr

[a] After 48 hr of incubation at 27°C.

uninoculated sirup remained buddy and unpalatable. After 72 hr of growth the untreated dilute sirup was thick and could be poured only with difficulty.

Initially, sirup was diluted arbitrarily with 3 volumes of water to reduce the concentration of sucrose. Inasmuch as *Ps. geniculata* grows in this dilute sirup and removes the buddy flavor, it would be uneconomical to dilute the sirup further. A more concentrated sirup solution, however, would be preferable, provided it allowed the growth and activity of the inoculated organisms. Heated sirup was divided into two portions and diluted with 2 volumes and 3 volumes of cold water to give solutions of approximately 29 and 20° Brix, respectively. Table 3 shows the results of inoculating these dilute sirups with *Ps. geniculata* and incubating at 27°C. After 24 hr there was no buddy flavor in the sirup diluted with 3 volumes of water. Although the flavor in the sirup diluted with 2 volumes of water was better than that of the uninoculated sirup, it required an additional 24 hr of incubation to completely remove all buddiness.

Table 3. Effect of concentration of dilute buddy sirup on *Ps. geniculata* activity in removing buddy flavor.

Treatment[a]	Time[b]	Observation
Diluted 1:3	24 hr	Buddy flavor not completely gone
	48 hr	Buddy flavor gone
Diluted 1:4	24 hr	Buddy flavor gone
	48 hr	Buddy flavor gone

[a] Buddy sirup heated 5 min at boiling and added to the required quantity of cold water.
[b] Incubation at 27°C.

Small-scale pilot-plant studies of the controlled fermentation of dilute buddy maple sirup by *Ps. geniculata* 4 were undertaken in the plant of a maple producer under normal operating conditions. One hundred and fifty gallons of buddy maple sirup were heated to boiling in a sirup finishing pan heated with high-pressure steam. The sirup was passed through the pan continuously, and it was assumed that the hold-up time in the pan was sufficient to allow the sirup to remain at the boiling temperature at least 5 min. The hot sirup was pumped into a tank truck containing approximately 500 gallons of cold water. When all the sirup was in the tank and thoroughly mixed, the temperature was 29°C and sugar concentration was 21° Brix. The sirup solution was inoculated with a heavy suspension of *Ps. geniculata* and left to incubate. After 24 hr a sample was removed from the tank and evaporated to standard-density sirup. The product was medium amber in color, having none of the off-flavor or buddiness present in the original sirup. It was found, however, that the entire tank of solution had become so viscous that quantities of material could be scooped out.

The great increase in viscosity in the dilute sirup after heat treating the original sirup was unexpected. This could be due to either: 1) insufficient heat treatment of the sirup, or 2) contamination from the tank. The latter appears more plausible because, although the tank had been rinsed well with water before use, no particular precautions were taken to sanitize it. There were seams and corners in the tank that could have served as foci of contamination.

The experiments were repeated with several smaller containers (55-gallon drums and 500-gallon stock tanks) sanitized as described in the Procedures. Either 25- or 35-gallon quantities of buddy sirup were heated continuously at the boiling point in the finishing pan, and then added to 75 or 105 gallons of cold water in the stock tanks to give a 1:3 dilution of sirup. For the experiments in the 55-gallon drum, 10 or 12 gallons of sirup were heated in the finishing pan as a batch, and then transferred to 30 or 36 gallons of cold water. When the temperatures of the diluted sirups reached approximately 26°C they were inoculated with *Ps. geniculata* and incubated at room temperature. After 24 hr of incubation none of the solutions were viscous or even appeared as though they were about to become viscous. The solutions were concentrated to standard-density sirup. In all instances the flavor, although not completely free of buddiness, was of an acceptable level, in contrast to the flavor of the original sirup. The 24-hr incubation period was not long enough for complete disappearance of the buddy flavor; 36 or 48 hr would have been more satisfactory. The course of the fermentation and the disappearance of the buddy flavor should be followed at intervals by reconcentrating samples of the fermenting solutions.

Amino acid composition. The nature of the component(s) of buddy sap responsible for the buddy flavor in sirup has not been determined. However, it has been established (unpublished data) that while normal sirup contains only a trace (a fraction of a ppm) amino nitrogen it is increased 40-fold in buddy sirup. It was of interest to determine whether the action of the *Pseudomonas* in eliminating the buddy flavor of sirup involved the nitrogen contents of the sirup. Heated buddy sirup, diluted 1:3 with water, was fermented for 48 hr with a suspension of *Ps. geniculata,* then reconcentrated to normal-density sirup. The buddy flavor had disappeared. A sample of the fermented sirup and a sample of the original sirup were submitted for amino acid analysis by the method of Spackman *et al.* (1958), modified by Zacharius and Talley (1962), using an automatic amino acid analyzer. The results (Table 4, Fig. 1) show differences in the amino acid concentrations between the two sirups.

Table 4. Concentrations of amino acids in buddy sirup before and after fermentation with *Ps. geniculata.*

Amino acid	Buddy sirup (moles/ml)	Treated sirup[a] (moles/ml)	Treated/Buddy × 100
Aspartic acid	.7608	.1743	18
Threonine	.1963		
Asparagine, Glutamine	.5091	.4485	88
Proline	.4942	.2723	55
Glutamic acid	.8195	.7130	87
Glycine	1.5990	1.6472	92
Alanine	.1680		
Valine	.3627	.1818	50
Methionine	.0493	.0153	31
Isoleucine	.2899	.1219	42
Leucine	.1885	.0082	4
Unknown peak	.3785	.1689	44

[a] Fermented with *Ps. geniculata* for 48 hr at 27°C.

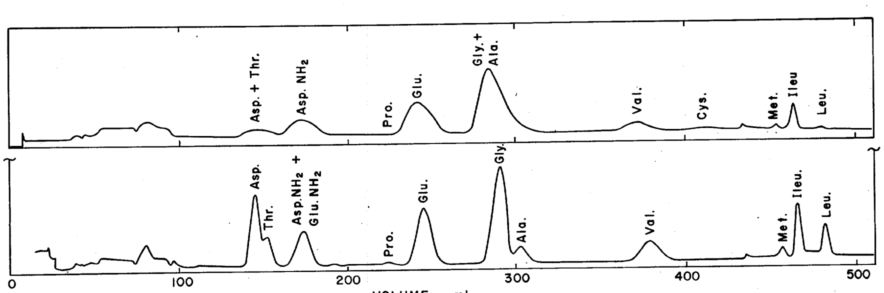

Fig. 1. Elution curve for amino acid from buddy sirup before and after fermentation with *Ps. geniculata.* Top: Sirup after fermentation. Bottom: Sirup before fermentation.

In general, the amino acid concentrations were lower in the fermented sirup than in the untreated sirup. Several of the amino acids were markedly reduced: leucine was reduced 96%, methionine 70%, and aspartic acid and methionine 82%. About half of the isoleucine, valine, and proline were removed from the sirup through the action of the bacteria. Asparagine-glutamine, glutamic acid, and glycine-alanine were the amino acids apparently least utilized by the cells, since only 9–13% disappeared. Fig. 1, however, shows two distinct glycine-alanine peaks in untreated sirup. In the fermented buddy sirup there was only one broad peak with approximately the same area as the two peaks, hence the apparent similarity in amino acid concentration in the two sirups. It has not been determined whether the alanine alone disappeared or whether the material in the fermented sirup was a new complex which was eluted in the same position as glycine-alanine in the untreated sirup.

Although the decrease in amino acid concentration in buddy sirup as a result of *Ps. geniculata* growth correlated with the disappearance of the buddy flavor, further work is required before the undesirable flavor of buddy sirup can be related to greater amino acid concentrations.

Slime formation in buddy sirup. Cold sirup diluted with 3 volumes of water and allowed to stand at room temperature became viscous and ropy or slimy. On reconcentration to standard-density sirup, the ropiness persisted and the sirup was definitely of an inferior grade. A gram-positive dipplococcus organism was isolated from the original sirup and in large numbers from the viscous dilute sirup. The organisms have been tentatively identified as *Leuconostoc mesenteroides* by standard bacteriological procedures (Bergey, 1957). It is interesting to note that on TGE agar plates the pinpoint colonies consist of typically coccal organisms, but in smears prepared from viscous or slimy dilute buddy sirup the dipplococci are surrounded by a slime layer so they appear to be gram-negative rod-like organisms with strongly gram-positive polar bodies.

In earlier studies on ropy maple sirup (Fabian and Buskirk, 1935) a number of organisms were isolated from maple sap that was later made into ropy sirup. None of the isolated organisms could produce ropiness or slime formation when inoculated into normal sirup. Thus the condition of ropiness appears to be developed by the growth of the organisms in sap. Isolation of *L. mesenteroides* from the buddy sirup, however, indicates the occurrence of contamination after the sirup is made. In processing maple sap to sirup, the solution is concentrated from 2% to 66% sugar at temperatures ranging from 100 to 104°C for approximately 90 min, which would effectively destroy vegetative forms of microorganisms.

The presence of *L. mesenteroides* in the buddy sap indicated that this organism may be a normal contaminant of the sugarhouse and siruping equipment. However, ropy or viscous sap is observed only late in the maple season. Regular maple sirup and sap apparently are not affected by this organism. This was demonstrated by inoculating sterile normal and buddy sirups, each diluted with 3 volumes of sterile water, with a culture of *L. mesenteroides*. After two days of incubation at room temperature (27°C), the dilute buddy sirup culture was viscous and slimy whereas the dilute normal sirup showed no signs of thickening. The latter culture was observed for an additional several days but no increase in viscosity was noted.

In another experiment, growth of *L. mesenteroides* was compared on normal and buddy sap. Buddy sirup was diluted with water to a sugar concentration of 3° Brix to simulate sap. Twenty-five-ml samples of sterile normal or buddy sap in 125-ml Erlenmyer flasks were inoculated with a quantity of concentrated cells sufficient to just show turbidity. After 24 hr of incubation at 29°C the optical density and viable bacterial count were determined, as shown in Table 5. In the normal sap there was only a small increase in the number of cells, whereas in buddy sap there was a 30-fold increase. The optical density of the buddy sap was considerably greater than that of the control or the normal sap. Some of this turbidity was due to the organisms present, but a portion was undoubtedly caused by the polysaccharide or slime produced by the bacteria. This formation of turbidity, or opalescence, may explain the appearance of "milky" sap often encountered in the field by the maple producer, particularly in warm weather.

Table 5. Growth of *L. mesenteroides* and *Ps. geniculata* in normal and buddy sap.

Sap	*L. mesenteroides* O.D.[a]	*L. mesenteroides* Cell count[b]	*Ps. geniculata* Cell count[b]
Buddy	.340	4.7×10^8/ml	1.29×10^9/ml
Normal	.019	4.1×10^7	3.44×10^9
Control (0 hr)	.010	1.4×10^7	2.14×10^6

[a] Optical density at 540 mμ.
[b] After 24 hr of incubation at 27°C.

Since *L. mesenteroides* grows more rapidly in buddy sap than in normal sap, presumably at the expense of the increased nitrogen concentration in buddy sap, it was of interest to determine whether *Ps. geniculata* growth rate was also stimulated. The results of such an experiment are shown in Table 5. The *Ps. geniculata* population was somewhat greater in the normal sap than in buddy sap; optical densities were not obtained, because the turbidity in both sets of flasks was just faintly visible.

The increased nitrogen concentration in buddy sap and sirup leads to the assumption that these would be better media for the growth of bacteria—both for increased growth rate and for the total population of cells that could be supported. This is true for the growth of *L. mesenteroides,* but *Ps. geniculata,* although the cells do metabolize away the factor responsible for the buddy characteristic, appears to prefer the normal sap for growth. This may indicate the presence in buddy sap of another factor that may retard the growth of the organism in a medium containing a more favorable nitrogen-carbon balance.

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Ms. rec'd 1/27/64.

The authors thank Dr. Eugene A. Talley for carrying out the determinations of the amino acids in fermented and unfermented buddy maple sirup.